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An immunofluorescent survey of the brown tide chrysophyte *Aureococcus anophagefferens* along the northeast coast of the United States

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Abstract. Surveys were conducted along the northeast coast of the USA, between Portsmouth, NH, and the Chesapeake Bay in 1988 and 1990, to determine the population distribution of *Aureococcus anophagefferens*, the chrysophyte responsible for massive and destructive 'brown tides' in Long Island and Narragansett Bay beginning in 1985. A species-specific immunofluorescent technique was used to screen water samples, with positive identification possible at cell concentrations as low as 10–20 cells ml^{-1}. Both years, *A.anophagefferens* was detected at numerous stations in and around Long Island and Barnegat Bay, NJ, typically at high cell concentrations. To the north and south of this 'center', nearly half of the remaining stations were positive for *A.anophagefferens*, but the cells were always at very low cell concentrations. Many of the positive identifications in areas distant from Long Island were in waters with no known history of harmful brown tides. The species was present in both open coastal and estuarine locations, in salinities between 18 and 32 practical salinity units (PSU). The observed population distributions apparently still reflect the massive 1985 outbreak when this species first bloomed, given the number of positive locations and high abundance of *A.anophagefferens* in the immediate vicinity of Long Island. However, the frequent occurrence of this species in waters far from this population 'center' is disturbing. *Aureococcus anophagefferens* is more widely distributed than was previously thought. Numerous areas thus have the potential for destructive brown tides such as those associated with the sudden appearance of the species in 1985.

Introduction

In 1985, a massive phytoplankton bloom termed the 'brown tide' occurred in the coastal waters and bays of Long Island, Rhode Island and New Jersey (Cosper *et al.*, 1989a). In some of these areas, cell concentrations were so high that the water became dark brown, limiting light penetration to the extent that large expanses of eelgrass (*Zostera marina*) were destroyed (Dennison *et al.*, 1989). Equally devastating was the effect of this bloom on shellfish, especially scallops and mussels, which experienced massive recruitment failure and mortality (Tracey, 1988; Bricelj and Kuenstner, 1989). Smaller but similar brown tide blooms have recurred in Long Island nearly every year since this initial outbreak, but not in other locations.

The causative organism is a previously undescribed chrysophyte named *Aureococcus anophagefferens* Hargraves et Sieburth (Sieburth *et al.*, 1988). Retrospective examination of archived samples using the transmission electron microscope (TEM) have shown that this species was present in very low abundance in Narragansett Bay at least 3 years before the 1985 brown tide (Sieburth and Johnson, 1989). These authors argued that *A.anophagefferens* is a natural but previously unnoticed component of the picoplankton, existing at low background concentrations that increased to bloom levels in 1985 in response to

exceptional, and as yet unknown, growth conditions. Reduced rainfall, elevated salinities, the delivery of specific micronutrients and reduced grazing pressure have been suggested as causative factors leading to the spectacular blooms (Cosper *et al.*, 1989b).

Aureococcus anophagefferens is very small (~2 μm diameter) and lacks morphological features which distinguish it from similar sized picoplankters using either phase-contrast or epifluorescence microscopy. TEM techniques could be used for positive identification, but are not practical for most field studies. It has thus been difficult to identify and count *A.anophagefferens* in mixed plankton assemblages unless it is present at high cell concentrations relative to similar sized, co-occurring species. Accordingly, little is known of the population dynamics of this species or of its geographic distribution beyond the Long Island embayments where its blooms have been most prominent and persistent.

The development of a species-specific antibody to the outer cell wall proteins of *A.anophagefferens* (Anderson *et al.*, 1989) has done much to change this situation. Using indirect immunofluorescent techniques, this antibody can be used to screen cultures or plankton samples quickly and accurately. At suitable antibody dilutions, no cross-reactions have been observed with 46 phytoplankton cultures representing five algal classes, including 20 species from the class Chrysophyceae. It is thus possible to positively identify and count *A.anophagefferens* at cell concentrations as low as 10–20 cells ml^{-1}. Here we report the use of this new technique in a survey of the population distribution of *A.anophagefferens* in coastal waters between New Hampshire and Virginia.

Method

Cultures and experimental design

For studies of preservation effects and counting method intercalibration, cultures of *A.anophagefferens* (clone BP3B, obtained from E.M.Cosper) were maintained in K medium (Keller and Guillard, 1985) at 20°C at 250 μE m^{-2} s^{-1} on a 14:10 h light:dark cycle.

Immunofluorescent identification and counting

The general protocol for immunofluorescent labeling of *A.anophagefferens* cells was that given by Anderson *et al.* (1989). Modifications included the use of 1.0 instead of 0.2 μm black polycarbonate filters. This significantly decreased the sample processing time without loss of cells. Another change was that a drop of 9:1 glycerol:phosphate-buffered saline (PBS) was smeared on the coverslip before it was placed over the filter. This more evenly distributed the sample on the filter. For all samples, an antibody dilution of 1:3200 was used. This concentration is low enough to eliminate cross-reactions, but sufficient for *A.anophagefferens* cells to be easily identified by their fluorescent 'halo'. A volume of 1–2 ml was typically processed and 50–60 fields counted on the filter at 400× magnification, resulting in an estimated detection limit of 10–20 cells

ml^{-1}. When a survey sample was positive for *A.anophagefferens*, but the cell concentration was very low, a second subsample was processed and analyzed for confirmation.

Survey details

Between 19 July and 20 September, 1988, 81 water samples were taken from Portsmouth, New Hampshire, to Manahawkin, New Jersey, at depths of 0–5 m (Table I, Figure 1). A well-mixed subsample of each sample was poured into 15 ml polypropylene centrifuge tubes containing 0.13 ml cold 70% glutaraldehyde (0.6% glutaraldehyde final concentration). These were kept on ice in the field and then stored at 4°C in a laboratory refrigerator. In 1990, 65 locations

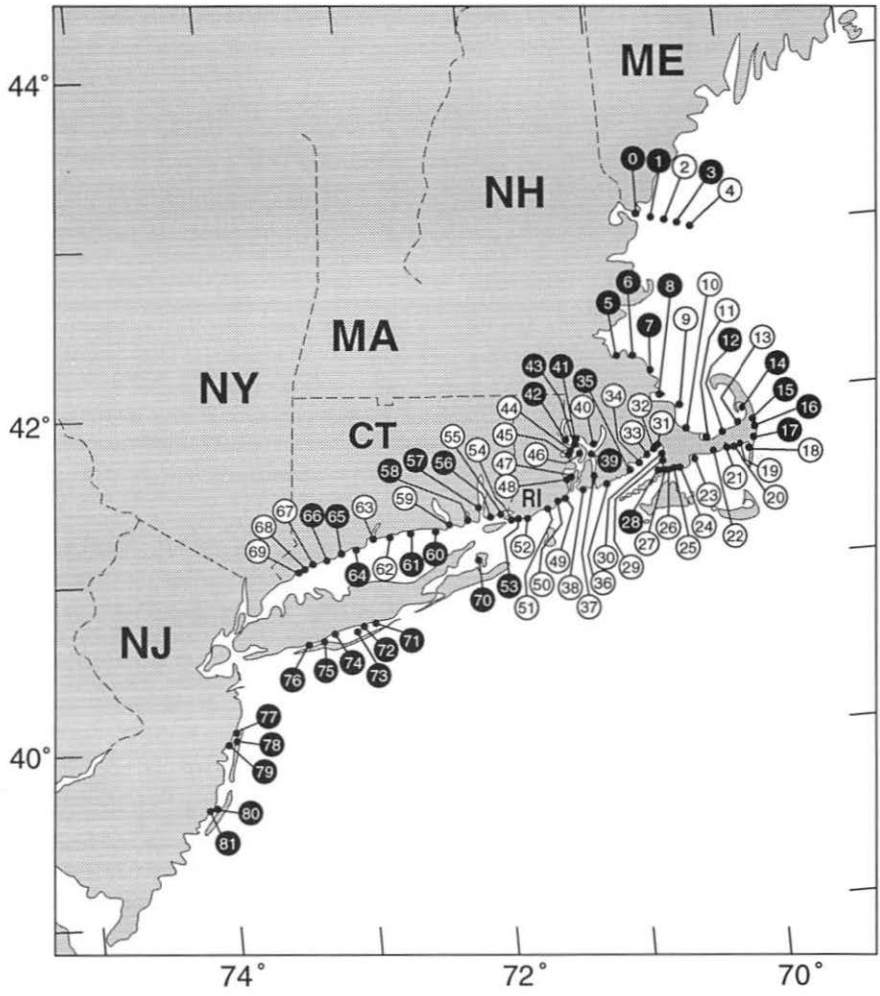

Fig. 1. Stations sampled in the 1988 survey for *A.anophagefferens*. Exact coordinates of the stations are given in Table I. Black circles denote positive identification of *A.anophagefferens*.

Table I. 1988 survey results

Station	Cross-ref. station	Date (month/day/year)	Location	Latitude	Longitude	Salinity (PSU)	Cells ml^{-1}	+/−
New Hampshire								
0	WHOI CA-000	08/3/88	Gulf of Maine	43 04 28.1	70 43 37.5	26.9	35	+
1	WHOI CA-001	08/3/88	Gulf of Maine	43 03 30.2	70 39 57.7	30.2	119	+
2	WHOI CA-002	08/3/88	Gulf of Maine	43 02 52.3	70 35 15.9	30.7	0	−
3	WHOI CA-003	08/3/88	Gulf of Maine	43 02 05.6	70 30 09.8	30.8	15	+
4	WHOI CA-004	08/3/88	Gulf of Maine	43 00 54.9	70 23 15.7	30.9	0	−
Massachusetts								
5	WHOI HO-001	09/03/88	Hingham Harbor, Hull	42 15 44.7	70 53 38.6	19.0	40	+
6	WHOI CH-001	09/03/88	Little Harbor, Cohasset	42 14 56.0	70 47 57.0	19.7	10	+
7	WHOI SU-001	09/03/88	Scituate Harbor, Scituate	42 11 56.8	70 43 37.6	18.0	13	+
8	WHOI PM-001	09/03/88	Plymouth Bay, Duxbury	42 02 00.0	70 40 00.0	18.0	12	+
9	WHOI SG-001	09/03/88	Cape Cod Bay, Plymouth	41 50 59.0	70 31 40.0	16.5	0	−
10	WHOI SD-001	07/20/88	Mill Creek, Sandwich	41 45 49.0	70 29 00.0	18.0	0	−
11	WHOI HA-001	07/20/88	Barnstable Harbor, Hyannis	41 42 25.7	70 17 59.5	21.0	0	−
12	WHOI DN-001	07/20/88	Sesuit Harbor, Dennis	41 45 13.0	70 09 08.2	19.5	13	+
13	WHOI OL-001	07/20/88	Rock Harbor, Orleans	41 48 00.4	70 00 25.7	24.0	0	−
14	WHOI WF-001	07/20/88	Wellfleet Harbor, Wellfleet	41 55 32.0	70 02 08.9	24.5	147	+
15	WHOI OL-003	07/20/88	Salt Pond, Orleans	41 50 08.1	69 58 21.0	21.5	22	+
16	WHOI OL-002	07/20/88	Town Cove, Orleans	41 47 17.8	69 59 09.6	23.2	697	+
17	WHOI CM-002	07/20/88	Pleasant Bay, Chatham	41 43 46.3	69 59 31.5	23.0	52	+
18	WHOI CM-001	07/20/88	Stage Harbor, Chatham	41 39 59.7	69 58 11.2	23.0	0	−
19	WHOI HW-001	07/20/88	Wechmere Harbor, Harwich	41 40 00.0	70 03 48.0	25.0	0	−
20	WHOI DN-002	07/20/88	Bass River, Dennis	41 40 00.0	70 10 32.0	27.5	0	−
21	WHOI HA-002	07/20/88	Lewis Bay, W. Yarmouth	41 38 37.0	70 15 14.0	30.0	0	−
22	WHOI CT-002	07/20/88	Cotuit Bay, Barnstable	41 36 51.0	70 25 54.0	29.5	0	−
23	WHOI CT-001	07/20/88	Popponesset Bay, Mashpee	41 35 08.0	70 27 47.0	28.8	0	−
24	WHOI FM-003	07/20/88	Waquoit Bay, Falmouth	41 33 59.0	70 30 51.0	29.5	0	−
25	WHOI FM-002	07/20/88	Waquoit Bay, Falmouth	41 34 05.0	70 31 34.0	28.8	0	−
26	WHOI FM-001	07/20/88	Green Pond, Falmouth	41 33 43.0	70 34 01.0	26.5	0	−
27	WHOI FM-004	08/18/88	Falmouth Harbor, Falmouth	41 32 53.0	70 36 07.0	29.9	0	−
28	WHOI WH-002	09/03/88	Snug Harbor, Falmouth	41 36 57.0	70 38 06.0	30.0	20	+
29	WHOI PS-002	09/03/88	Phinneys Harbor, Bourne	41 42 49.0	70 36 57.0	30.0	0	−

30	WHOI PS-001	09/03/88	Red Brook Harbor, Bourne	41 40 34.0	70 36 51.0	30.0	0	–
31	WHOI SG-002	09/03/88	Buttermilk Bay, Wareham	41 44 45.0	70 37 15.0	29.8	0	–
32	WHOI OS-001	09/03/88	Wareham River, Wareham	41 45 00.0	70 42 30.0	23.2	0	–
33	WHOI MR-001	09/03/88	Sippican Harbor, Marion	41 42 11.0	70 45 03.0	30.3	0	–
34	WHOI SN-001	09/03/88	Nasketucket Bay, Fairhaven	41 35 30.0	70 51 00.0	30.4	0	–
35	WHOI NB-001	09/03/88	Apponagansett Bay, Dartmouth	41 35 00.0	70 57 48.0	30.5	20	+
36	WHOI WP-001	09/03/88	Westport Harbor, Westport	41 30 50.0	71 04 45.0	30.5	0	–
Rhode Island								
37	WHOI TR-001	09/15/88	Sakonnet River, Portsmouth	41 33 08.0	71 13 58.0	30.0	0	–
38	WHOI NW-022	09/15/88	Goose Neck Cove, Newport	41 27 09.0	71 20 33.0	29.8	0	–
39	WHOI PI-001	09/15/88	Narragansett Bay, Portsmouth	41 35 27.0	71 16 17.0	30.0	10	+
40	WHOI BT-001	09/15/88	Mount Hope Bay, Bristol	41 40 00.0	71 15 00.0	29.5	0	–
41	WHOI BG-001	09/15/88	Warren River, Warren	41 43 30.0	71 17 15.0	28.5	11	+
42	EPA 001	09/15/88	Narragansett Bay, Conimicnt Point	41 43 00.0	71 21 00.0	27.9	100	+
43	EPA 002	09/15/88	Narragansett Bay, Ohio Ledge	41 41 00.0	71 20 00.0	29.5	100	+
44	EPA 005	09/15/88	Narragansett Bay, Greenwich Bay	41 41 00.0	71 25 00.0	29.0	0	–
45	EPA 006	09/15/88	Narragansett Bay, Greenwich Cove	41 40 00.0	71 26 00.0	30.1	0	–
46	EPA 003	09/15/88	Narragansett Bay, Prudence Island	41 38 00.0	71 21 00.0	29.0	0	–
47	EPA 004	09/15/88	Narragansett Bay, Kingston	41 21 00.0	71 25 00.0	27.3	0	–
48	WHOI NP-002	09/15/88	Pettaquamscutt River, Narragansett	41 27 30.0	71 26 55.0	21.0	0	–
49	WHOI NP-001	09/15/88	Point Judith Pond, Narragansett	41 23 22.0	71 29 34.0	29.5	0	–
50	WHOI KS-001	09/15/88	Point Judith Pond, South Kingston	41 23 07.0	71 31 26.0	27.9	0	–
51	WHOI QH-001	09/15/88	Ninigret Pond, Charlestown	41 20 48.0	71 41 40.0	27.3	0	–
52	WHOI WL-003	09/15/88	Quonochontaug Pond, Westerly	41 20 08.0	71 44 00.0	30.2	0	–
53	WHOI WL-002	09/15/88	Winnapog Pond, Westerly	41 20 05.0	71 46 22.0	30.0	38	+
54	WHOI WL-001	09/15/88	Pawcatuck River, Westerly	41 19 35.0	71 50 28.0	26.0	0	–
Connecticut								
55	WHOI MS-001	09/14/88	Mystic Harbor, Mystic	41 20 52.0	71 57 48.0	28.5	0	–
56	WHOI NL-002	09/14/88	Poquonock River, Groton	41 20 10.0	72 02 00.0	28.0	22	+
57	WHOI NL-001	09/14/88	Thames River, New London	41 20 53.0	72 05 56.0	28.0	53	+
58	WHOI NN-001	09/14/88	Niantic River, East Lyme	41 19 35.0	72 10 31.0	28.8	306	+
59	WHOI OY-001	09/14/88	Connecticut River, Old Saybrook	41 17 14.0	72 21 34.0	13.0	0	–
60	WHOI CN-001	09/14/88	Clinton Harbor, Clinton	41 16 07.0	72 31 37.0	27.3	64	+
61	WHOI GF-001	09/14/88	Guilford Harbor, Guilford	41 16 15.0	72 40 00.0	27.0	109	+
62	WHOI BN-001	09/14/88	Branford Harbor, Branford	41 15 43.0	72 48 55.0	26.5	0	–

Table I. Continued

Station	Cross-ref. station	Date (month/day/year)	Location	Latitude	Longitude	Salinity (PSU)	Cells ml^{-1}	+/−
63	WHOI NH-001	09/14/88	New Haven Harbor, New Haven	41 16 53.0	72 55 38.0	25.5	0	−
64	WHOI MF-001	09/14/88	Milford Harbort, Milford	41 13 06.0	73 03 19.0	25.5	100	+
65	WHOI BP-001	09/14/88	Bridgeport Harbor, Bridgeport	41 10 24.0	73 09 32.0	25.5	9	+
66	WHOI SW-001	09/14/88	Sherwood Mill Pond, Westport	41 07 05.0	73 20 15.0	25.2	20	+
67	WHOI NW-002	09/14/88	Norwalk Harbor, Norwalk	41 05 45.0	73 24 19.0	26.5	0	−
68	WHOI NW-001	09/14/88	Holly Pond, Norton	41 03 03.0	73 29 18.0	27.0	0	−
69	WHOI SF-001	09/14/88	Stamford Harbor, Stamford	41 02 20.0	73 32 45.0	21.0	0	−
New York								
70	SUNY WNB	07/21/88	West Neck Bay, Shelter Island	41 03 48.0	72 20 40.0	27.0	1550	+
71	EPA GSB#4	07/19/88	Carmans River, Brookhaven	40 45 20.0	72 53 34.0	24.1	2915	+
72	SUNY BP	07/20/88	Patchogue Bay, Blue Point	40 44 16.0	73 02 06.0	23.0	1400	+
73	EPA GSB#3	07/19/88	Patchogue Bay, Blue Point	40 43 03.0	73 01 00.0	25.3	3871	+
74	SUNY 1M	07/20/88	Great South Bay, Islip	40 42 22.0	73 11 18.0	24.0	2500	+
75	EPA GSB#2	07/19/88	Great South Bay, Islip	40 40 54.0	73 16 52.0	28.6	1634	+
76	EPA GSB#1	07/19/88	Great South Bay, Lindenhurst	40 39 50.0	73 21 10.0	23.3	1572	+
New Jersey								
77	NJDEP 1	09/20/88	Barnegat Bay at Mantoloking	40 03 30.0	74 02 30.0	NA	784	+
78	NJDEP 2	09/20/88	Barnegat Bay at Lavallette	39 58 30.0	74 05 00.0	NA	146	+
79	NJDEP 3	09/20/88	Barnegat Bay at Toms River	39 56 00.0	74 06 45.0	NA	204	+
80	NJDEP 5	09/20/88	Barnegat Bay at Surf City	39 41 00.0	74 10 30.0	NA	34900	+
81	NJDEP 4	09/20/88	Barnegat Bay at Manahawkin	39 41 00.0	74 12 00.0	NA	141000	+

NA, not available.

Table II. 1990 survey results

Station	Cross-ref. station	Date (month/day/year)	Location	Latitude	Longitude	Salinity (PSU)	Cells ml^{-1}	+/−
Massachusetts								
1	WHOI HL-001	09/12/90	Hingham Harbor, Hull	42 15 44.7	70 53 38.6	28.8	81	+
2	WHOI CH-001	09/12/90	Little Harbor, Cohasset	42 15 08.6	70 47 43.8	28.9	0	−
3	WHOI SU-001	09/12/90	Scituate Harbor, Scituate	42 11 56.8	70 43 37.6	29.2	0	−
4	WHOI PM-001	09/12/90	Plymouth Bay, Duxbury	42 02 00.0	69 40 00.0	28.5	49	+
5	WHOI SD-001	09/12/90	Mill Creek, Sandwich	41 45 51.6	70 29 09.9	28.5	0	−
6	WHOI HA-001	09/11/90	Barnstable Harbor, Hyannis	41 42 25.7	70 17 59.5	21.5	0	−
7	WHOI DN-001	09/11/90	Sesuit Harbor, Dennis	41 45 13.0	70 09 08.2	25.0	16	+
8	WHOI OL-001	09/11/90	Rock Harbor, Orleans	41 48 00.4	70 00 25.7	24.7	0	−
9	WHOI WF-001	09/11/90	Wellfleet Harbor, Wellfleet	41 55 32.0	70 02 08.9	29.5	65	+
10	WHOI PV-001	09/11/90	MacMillan Wharf, Provincetown	41 02 58.0	70 10 59.1	28.9	16	+
11	WHOI WF-002	09/11/90	Newcombe Hollow Beach, Wellfleet	41 57 49.6	69 59 44.1	29.2	16	+
12	WHOI OL-003	09/11/90	Salt Pond, Orleans	41 50 08.1	69 58 23.8	27.9	16	+
13	WHOI OL-002	09/11/90	Town Cove, Orleans	41 47 17.8	69 59 09.6	27.5	16	+
14	WHOI CM-002	09/11/90	Pleasant Bay, Chatham	41 43 46.3	69 59 31.5	27.0	16	+
15	WHOI CM-001	09/11/90	Stage Harbor, Chatham	41 39 59.7	70 58 11.2	27.5	49	+
16	EPA 027	08/05/90	SE of Monomoy Is., Nantucket Sound	41 31 30.0	70 04 18.6	31.6	0	−
17	EPA 029	08/05/90	W of Nantucket Is., Nantucket Sound	41 23 00.0	70 10 36.0	31.6	421	+
18	EPA 037	08/16/90	South of Hyannisport, Nantucket Sound	41 31 18.0	70 17 37.8	31.7	0	−
19	EPA 038	08/16/90	SE of Cape Poge, Martha's Vineyard, Nantucket Sound	41 22 47.4	70 23 53.4	31.8	16	+
20	EPA 068	08/06/90	NW of Gay Head, Martha's Vineyard, Vinyard Sound	41 22 16.8	70 50 27.0	NA	130	+
21	EPA 099	08/06/90	New Bedford Harbor	41 38 33.0	70 54 42.0	NA	16	+
Rhode Island								
22	EPA 070	08/07/90	Narraganset Bay	41 38 28.8	71 18 00.6	30.1	32	+
23	URI II	05/07/90	Narraganset Bay	41 34 07.0	71 23 00.0	28.3	16	+
24	URI II	06/04/90	Narraganset Bay	41 34 07.0	71 23 00.0	29.6	1865	+
25	URI II	07/03/90	Narraganset Bay	41 34 07.0	71 23 00.0	28.3	227	+
26	URI II	07/16/90	Narraganset Bay	41 34 07.0	71 23 00.0	29.6	697	+

Table II. Continued

Station	Cross-ref. station	Date (month/day/year)	Location	Latitude	Longitude	Salinity (PSU)	Cells ml^{-1}	+/–
Connecticut								
27	EPA 107	08/14/90	Block Island Sound	41 19 30.0	71 58 36.0	29.8	0	–
28	EPA 106	08/14/90	Mystic River	41 21 52.8	71 57 52.2	28.8	0	–
29	EPA 077	08/13/90	Long Island Sound	41 10 59.4	72 29 09.6	28.5	16	+
30	EPA 079	08/13/90	Long Island Sound	41 10 29.4	72 42 21.6	NA	0	–
31	EPA 022	08/13/90	Long Island Sound	41 09 58.2	72 55 33.0	27.2	0	–
32	EPA 098	08/20/90	Long Island Sound	41 09 34.8	73 12 37.2	26.0	113	+
New York								
33	EPA 078	08/18/90	Long Island Sound	41 02 19.8	72 35 03.6	27.1	5322	+
34	SCDHS FP119	07/10/90	West Neck Bay	41 03 48.0	72 20 40.0	26.9	480141	+
35	EPA 104	08/19/90	Peconic Bay	40 57 24.0	72 30 12.0	27.6	0	–
36	SCDHS SH160	07/31/90	Shinnecock Bay	40 50 35.0	72 30 20.0	29.9	107842	+
37	SCDHS WSB	07/27/90	West Shinnecock Bay	40 49 05.0	72 35 15.0	NA	52837	+
38	SCDHS EMB	07/27/90	East Moriches Bay	40 48 19.0	72 39 52.0	NA	126335	+
39	SCDHS GSB 120	08/08/90	Great South Bay	40 43 55.0	72 57 32.0	22.9	292	+
40	SCDHS GSB 130	08/08/90	Great South Bay	40 44 03.0	73 01 00.0	21.9	162	+
41	EPA 023	08/19/90	Great South Bay	40 44 27.0	72 59 52.2	21.6	761	+
42	SCDHS GSB 150	08/08/90	Great South Bay	40 41 50.0	73 04 53.0	23.7	1409	+
New Jersey								
43	NJDEP 4	08/29/90	Sandy Hook Bay	40 27 00.0	74 01 00.0	NA	16	+
44	NJDEP 5	08/29/90	Atlantic Ocean at Sea Girt	40 08 00.0	74 01 00.0	NA	49	+
45	NJDEP 6	08/29/90	Atlantic Ocean at Island Beach	39 51 00.0	74 05 00.0	NA	243	+
46	NJDEP 3	08/29/90	Barnegat Bay at Holly Park	39 53 00.0	74 07 00.0	NA	49	+
47	NJDEP 2	08/29/90	Barnegat Bay at Waretown	39 47 00.0	74 11 00.0	NA	97	+
48	NJDEP 1	08/29/90	Barnegat Bay at Manahawkin	39 41 00.0	74 12 00.0	NA	216	+
49	EPA 118	08/11/90	Great Bay	39 30 00.0	74 22 00.0	24.5	16	+
50	NJDEP 7	08/29/90	Atlantic Ocean at Atlantic City	39 21 00.0	74 26 00.0	NA	0	–
51	NJDEP 8	08/29/90	Delaware Bay	39 05 00.0	74 55 00.0	NA	0	–
52	NJDEP 9	08/29/90	Delaware Bay	39 05 00.0	75 00 00.0	NA	0	–

Delaware								
53	EPA 035	08/03/90	Delaware Bay	39 03 45.6	75 18 00.0	23.6	0	–
54	EPA 032	08/04/90	Delaware Bay	38 55 45.6	75 10 33.0	27.0	0	–
55	EPA 150	08/18/90	Delaware Coast	38 35 36.0	75 06 42.0	29.7	0	–
Maryland								
56	EPA 034	08/18/90	Atlantic Coast	38 04 22.2	75 16 31.8	32.9	0	–
57	EPA 114	08/06/90	Broad Creek, Chesapeake Bay	38 44 42.0	76 14 30.0	10.3	0	–
58	EPA 065	08/16/90	Chesapeake Bay off Little Choptank River	38 33 27.0	76 24 04.8	11.2	0	–
59	EPA 041	08/08/90	Tangier Sound, Chesapeake Bay	38 01 40.8	75 54 06.0	16.3	0	–
Virginia								
60	EPA 258	08/10/90	Atlantic Coast	37 17 58.8	75 50 00.0	31.7	0	–
61	EPA 054	08/09/90	York River Enrrance, Chesapeake Bay	37 09 12.6	76 11 36.6	22.0	0	–
62	EPA 046	08/04/90	Chesapeake Bay off Church Neck	37 27 01.8	76 01 42.6	17.0	0	–
63	EPA 053	08/03/90	Rappahannock River Entrance, Chesapeake Bay	37 34 58.8	76 09 09.0	NA	0	–
64	EPA 192	08/05/90	Rappahannock River near Tappahannock	37 57 54.0	76 52 01.8	3.7	0	–
65	EPA 200	08/05/90	Rappahannock River	38 12 01.2	77 15 06.0	0.1	0	–

NA, not available.

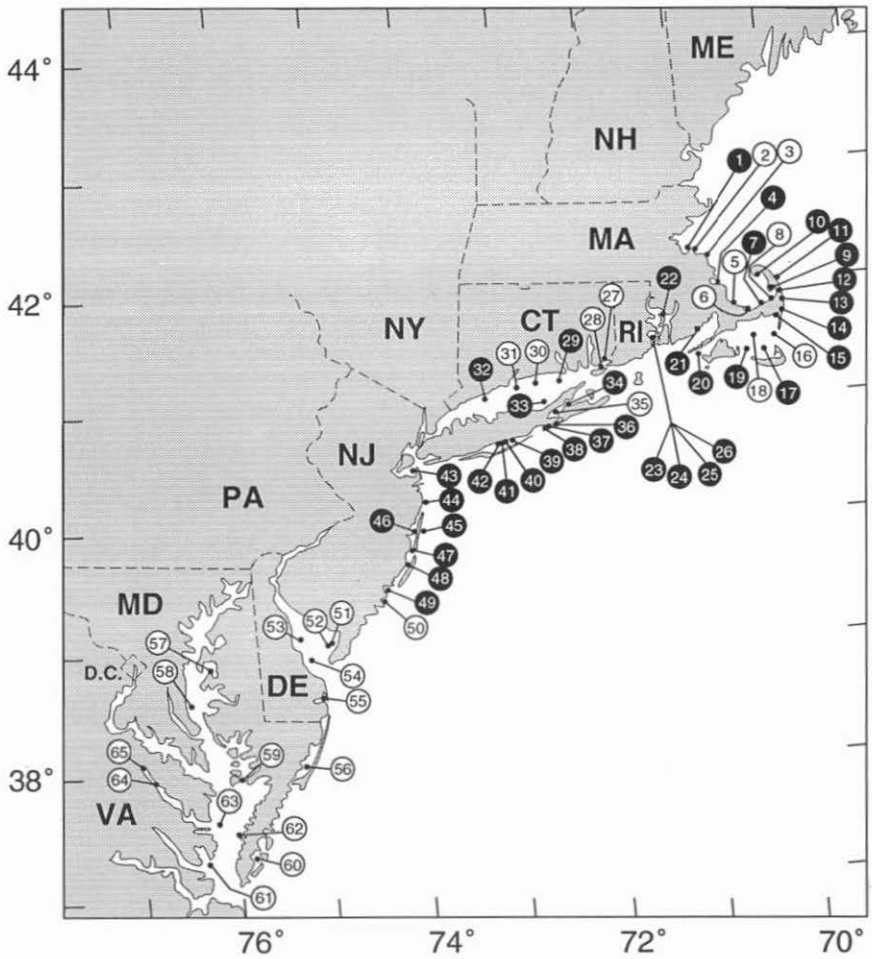

Fig. 2. Stations sampled in the 1990 survey for *A.anophagefferens*. Exact coordinates of the stations are given in Table II. Black circles denote positive identification of *A.anophagefferens*. Stations 23–26 are for the same location sampled on different dates.

were sampled (Table II, Figure 2). Stations with the prefix EPA were sampled as part of the US Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP). These samples were collected at the water surface and preserved with 0.6% glutaraldehyde. Samples with the prefix URI were also from surface waters, preserved in buffered formaldehyde. Other non-EMAP stations were sampled in 1988 and 1990 using methods described above for the 1988 survey. These are designated with the prefix WHOI (Woods Hole Oceanographic Institution), SCDHS (Suffolk County Department of Health Services), NJDEP (New Jersey Department of Environmental Protection) and SUNY (State University of New York at Stony Brook).

Intercalibration studies

Over an 8 month period in 1990–91, four glutaraldehyde (0.6%) preserved samples collected from Long Island embayments were counted by personnel from WHOI and SCDHS to determine counting variability with the immunofluorescent technique. Samples were first counted at Woods Hole by two technicians. A subsample of each of the four samples was then placed into new containers and sent to the SCDHS for re-quantification.

Previous attempts using laboratory-cultured material for the intercalibration failed, as cultured cells lysed quickly after dilution into either PBS, natural seawater or enriched seawater medium with and without glutaraldehyde. We now suspect that cultured *A.anophagefferens* cells (clone BP 3B) are extremely delicate and that researchers utilizing preserved material of this culture should be aware of this potential problem.

Results

Intercalibration studies

In an effort to better define the accuracy of the immunofluorescent method and the potential artifacts associated with the preservation and storage of samples, samples were counted independently at Woods Hole Oceanographic Institution and at the Suffolk County Department of Health. Atempts at interlaboratory calibration using diluted, preserved cultures gave misleading results since the concentration of cells known to be in the original culture differed substantially from subsequent immunofluorescent or phase-contrast cell counts. The losses were presumably due to either inadequate mixing of the samples (e.g. a pellet may not have been completely resuspended) or to inadequate preservation. Preliminary experiments have since shown that there was no difference in the counts between gently mixed samples and those that were shaken vigorously (data not shown). On the other hand, none of the preservatives tested [glutaraldehyde (0.6% and 2.5%), formalin (5%) or Lugol's] could maintain the initial cell concentration over time. Significant losses occurred within 1 week in laboratory cultures of *A.anophagefferens* and continued thereafter. Refrigeration appeared to slow the degradation process, but did not prevent it. In contrast, immunofluorescent counts of glutaraldehyde (0.6%)-preserved field samples were found to be constant over an extended period of time (6 months). Field samples were thus used for the intercalibration study.

Results of the intercalibration study are given in Table III. Considerable variability was observed between replicate counts by the same workers, especially at the two lowest cell concentrations (<1100 cells ml^{-1}) where coefficients of variation (CV) were 26–67%. At higher concentrations, the CVs were 10–30%. Counts by the two laboratories were in general agreement, typically within 10–20% of each other.

1988 survey

Aureococcus anophagefferens was present throughout the region sampled, both

Table III. Intercalibration cell counts using field samples containing *A.anophagefferens* [cells ml^{-1} (SD)]

Sample	WHOI count[a] (n = 3)	SCDHS count[b] (n = 5)
GSB-120	213 (142)	158 (47)
GSB-150	1083 (283)	772 (400)
WSB	84 957 (28 232)	77 904 (9818)
EMB	135 278 (40 509)	160 987 (16 251)

[a]Counts at Woods Hole Oceanographic Institution.
[b]Counts at SCDHS.

in estuaries and offshore waters (Table I, Figure 1). Positive identifications were less frequent and cell concentrations lower, however, in the north and south of the study area, compared to the Barnegat Bay, NJ, and Long Island, NY, region. Outside of this 'central' area, cell concentrations ranged from 9 to 697 cells ml^{-1}, with positive identification of *A.anophagefferens* in 28 of 70 samples (40%). Within Long Island and nearby Barnegat Bay, NJ, every sample tested (12 of 12) had *A.anophagefferens* cells, with concentrations ranging from 146 to 141 000 cells ml^{-1}.

1990 survey

Of the 65 stations sampled in 1990, 37 (56%) were positive for *A.anophagefferens* (Table II, Figure 2). The geographic distribution of *A.anophagefferens* extended from Boston, the most northern station in 1990, to southern New Jersey, with the highest concentrations (100 000–500 000 cells ml^{-1}) located in the shallow southern bays of Long Island. In Massachusetts waters, where there have been no previously recorded 'brown tide' outbreaks, two-thirds of the samples collected were positive, although most of these were just above the detection limit for the immunofluorescent technique. Positive samples were also recorded for the coastal waters of the Atlantic Ocean off Cape Cod (Station 11) and New Jersey (Stations 44–45), the open waters of Long Island Sound (Stations 29 and 33), and of Vineyard and Nantucket Sounds (Stations 17, 19 and 20) south of Cape Cod. No positive samples were recorded south of New Jersey, which included several samples from the Chesapeake Bay, the southernmost extent of the sampling.

Outside the Long Island area, cell concentrations in positive samples ranged from 16 to 1865 cells ml^{-1}, the latter being Station 24 in Narragansett Bay, RI. Samples at this same location were positive on four different occasions in May, June and July. For convenience, these are indicated as Stations 23–26 in Table II and Figure 2. Barnegat Bay, NJ, samples were all positive for *A.anophagefferens* in 1990, as was the case in 1988, but cell concentrations were much lower in 1990. Of the 13 stations throughout the entire study area that were sampled in both 1988 and 1990, 10 (77%) were positive both times for *A.anophagefferens*. Outside of Long Island and New Jersey, 46% of all stations sampled in 1990 were positive for *A.anophagefferens*.

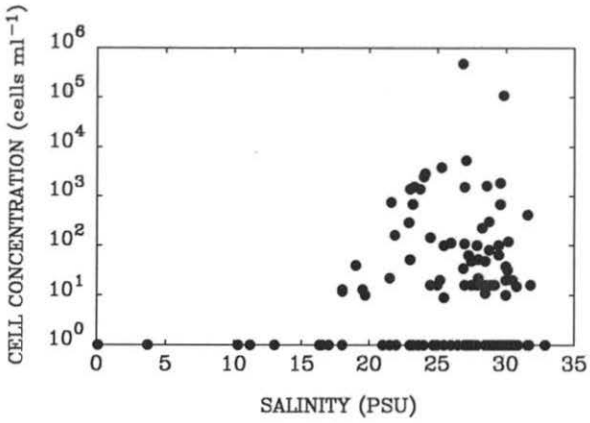

Fig. 3. Relationship between *A.anophagefferens* cell concentration and salinity (practical salinity units, PSU) for survey stations sampled in 1988 and 1990. Some stations listed in Tables I and II are not included because salinity measurements are not available.

Salinity tolerance

When possible, salinity was determined for the samples analyzed for *A.anophagefferens* abundance (Tables I and II). Results are summarized in Figure 3, which shows a broad salinity tolerance between 18 and 32 practical salinity units (PSU).

Discussion

The initial outbreak of *A.anophagefferens* in 1985 was a spectacular example of how the sudden growth and dominance of a single phytoplankton species can have devastating effects on a major ecosystem (Cosper *et al.*, 1989a). Earlier observations by Sieburth and Johnson (1989) demonstrate that this event is also an excellent example of how 'hidden flora' (species present at very low background concentrations) can emerge from obscurity and dominate the phytoplankton community. Given this history, it is indeed worrisome that the survey results reported here document the presence of *A.anophagefferens* in numerous locations where harmful brown tide outbreaks are unknown. In the years following the 1985 episode, only a few marine embayments on Long Island (and perhaps a few in New Jersey as well; Olsen, 1989) have been affected by recurrent blooms. In the future, outbreaks of this species could be more widespread if the exceptional environmental conditions that led to the 1985 blooms occur again, either regionally or locally.

Intercalibration study

The immunofluorescent method used here for *A.anophagefferens* has been used successfully with other picoplankters as well (Campbell and Carpenter, 1987; Shapiro *et al.*, 1989). Many of these organisms are quite fragile, however, and care must be taken to ensure that the fixation, storage and processing of samples

does not introduce artifacts that would make immunofluorescent cell counts inaccurate. In addition, cross-reactions and autofluorescence can introduce errors as well. A preliminary immunofluorescent study of *A.anophagefferens* by Anderson *et al.* (1989) suggested that formalin, glutaraldehyde and Lugol's iodine were all equally effective in preserving cells in field samples without significant cell loss, although glutaraldehyde maintained the best morphology and gave the brightest fluorescent labeling. Storage of samples for those experiments was of relatively short duration (~1 month), so long-term effects could not be assessed. Furthermore, since these samples were of natural plankton which included many morphologically similar picoplankton species, the initial abundance of *A.anophagefferens* prior to fixation and immunofluorescent counting was not known.

In the present study, an effort was made to compare counting results from two different laboratories to determine the degree of subjectivity in positive identifications made on the basis of immunofluorescence. Efforts to conduct the intercalibration with cultured *A.anophagefferens* cells failed, regardless of the preservative used, due to lysis of the cells. Decreases in cell concentration over time were observed in both phase-contrast and immunofluorescent counts. The decreases were thus not due to harsh processing (e.g. filtration) of samples for the immunofluorescent technique, but instead reflected gradual, unexplained lysis of cells during storage. This suggests caution in interpreting results from experiments using cultured *A.anophagefferens* cells (e.g. grazing experiments) in which samples are preserved and counted at a later date. We recommend that samples of cultured material be preserved, immediately refrigerated and counted the same day to prevent cell loss through time.

Preserved field samples were used in the intercalibration study after initial tests demonstrated that the *A.anophagefferens* cell concentrations in those samples remained constant through time. The durable or resistant cell walls of the 'wild cells' in field samples presumably reflect more suitable growth conditions than those in laboratory cultures.

Replicate counts within individual laboratories showed considerable variability at low cell concentrations, but precision more than doubled at higher concentrations (Table III). If needed, precision at low cell densities could be improved by processing a larger sample, or by counting more cells (or fields), recognizing that the distribution of cells on the filters was not uniform due to wall effects from the filter funnel. Counts by the two different laboratories were in good agreement, differing by ~10–20%. Immunofluorescent counts of *A.anophagefferens* can thus be consistent between laboratories and different investigators as long as the concerns described above for work with laboratory cultures are addressed, and that identification procedures are standardized to distinguish between autofluorescent and immunofluorescent cells.

Survey results

Surveys in both 1988 and 1990 depict a population distribution of *A.anophagefferens* centered around Long Island, with abundance and number of positive

locations decreasing to the north and south (Figures 1 and 2). This pattern is entirely consistent with the most recent series of brown tide blooms which have been conspicuous only in scattered bays of Long Island each year since 1986 (Cosper *et al.*, 1989b; Nuzzi and Waters, 1989; M.Waters, personal communication) and, to a lesser extent, in the Barnegat Bay area of New Jersey (Olsen, 1989). The species has undoubtedly been present in many other locations during these years judging from our survey results, but visible brown tide blooms, loss of eelgrass beds or mortality of shellfish following blooms have not been reported.

The simplest explanation for the population distribution we have documented is that *A.anophagefferens* is not a high-abundance member of the picoplankton community in the coastal waters of the study region. It remains relatively abundant only in those areas where it bloomed in years subsequent to the 1985 outbreak. In areas where the initial bloom occurred, but where major blooms have not recurred, the species has apparently diminished in number to the level of obscurity it had prior to 1985. A good example of such a location is Narragansett Bay, RI, where massive, brown water blooms of *A.anophagefferens* occurred in 1985 (Sieburth *et al.*, 1988), but where our survey detected only relatively low numbers of cells at several stations during the summer a few years later.

In other areas where we have detected the species in low concentrations, but where there have never been large brown water blooms, *A.anophagefferens* is simply a minor and inconspicuous member of the picoplankton community. Our data are insufficient to indicate whether these low 'background' cell concentrations are the result of supply from an external, dilute offshore source, as is suggested by the presence of cells in open coastal waters (stations 1 and 3 in Figure 1; stations 11, 17, 19, 44 and 45 in Figure 2), or whether *A.anophagefferens* is able to over-winter in the estuaries and bays. No life cycle information is available for this species, so the existence of dormant cysts that could survive through non-bloom periods in the sediments of shallow waters remains an open question. *Aureococcus anophagefferens* is tolerant of low temperatures, however, even though it grows better at 20–25°C (Cosper *et al.*, 1989b). When given sufficient time to adapt, growth of *A.anophagefferens* at 5°C is possible. These laboratory observations are consistent with the field results of Nuzzi and Waters (1989) who found *A.anophagefferens* cells to be present in Flanders Bay, Long Island, at concentrations of 100 000–300 000 cells ml^{-1} throughout the winter of 1987–88. Cells can thus over-winter in the plankton within estuaries and bays, and serve as an inoculum for future blooms, without the need for offshore resupply or dormant cyst stages.

What then is special about the areas of Long Island and Barnegat Bay where *A.anophagefferens* continues to bloom, and what was unusual about 1985 that allowed this species to bloom over a much larger area? One can only speculate in hindsight, of course, but Cosper *et al.* (1989b) suggest that the blooms did not spread from one central location to the others in 1985, but instead were a concurrent series of discrete events in response to common regional environmental conditions. For this hypothesis to be valid, *A.anophagefferens* had to be

broadly distributed throughout the region prior to the outbreaks, which is in fact what our surveys show is the case now. The stimulatory conditions of 1985 are thought to include reduced rainfall, elevated salinities, and reduced grazing pressure and flushing in enclosed bays, followed by the delivery of specific organic and inorganic micronutrients that allowed the species to grow with minimal losses (Cosper *et al.*, 1989b). These conditions, though not as favorable as they were in 1985, still persist to some extent in certain Long Island embayments, since major blooms have recurred there, but not elsewhere in the region.

A prominent factor in this context may be the relatively long residence time of water in these embayments (Hardy, 1976; Pritchard and Gomez-Reyes, 1986), which might allow the species to persist given its ability to grow at low, winter temperatures. Water residence time in 1985 may have been the longest in recent years, judging from mean sea level records (Vieira, 1989), which may explain the severity of the 1985 bloom relative to those in Long Island waters in succeeding years. Other areas which are more open and flushed more efficiently, such as Narragansett Bay, have not supported a significant *A.anophagefferens* population in the years after the initial outbreak.

The population distribution patterns depicted by our surveys might thus represent 'pre-1985' abundances of *A.anophagefferens* throughout the region (i.e. hidden flora *sensu* Sieburth and Johnson, 1989), with an enhanced population 'center' on Long Island that reflects recent bloom occurrences and some degree of local advective transport. If environmental conditions become favorable for this species throughout the region, as Cosper *et al.* (1989b) suggest was the case in 1985, the seed or inoculum populations are present to initiate widespread blooms once again. An alternative scenario would be that local conditions might favor the growth and dominance of this species on a much smaller scale in isolated locations far from Long Island. In either case, the potential clearly exists for future outbreaks of *A.anophagefferens*.

It remains to be determined whether *A.anophagefferens* is an estuarine, neritic, or even pelagic species. This chrysophyte is not evenly distributed throughout the study area, it becomes less abundant as one moves away from its Long Island 'center' (Figures 1 and 2), and it was not observed in any samples south of New Jersey or in $\geq$50% of our other samples. Its salinity tolerance is relatively broad, as it occurred between 18 and 32 PSU in our samples (Figure 3), and between 20 and 32 PSU in field samples analyzed by Cosper *et al.* (1989b). The species is widespread, but not necessarily cosmopolitan.

Detection limits and species specificity add obvious qualifications to these inferences. Our immunofluorescent method is capable of detecting cells at concentrations of 10–20 cells ml^{-1}, but we still cannot rule out the possibility that the species was present, but not detected in some samples. We must also acknowledge the possibility that the antibody could have cross-reacted with species other than *A.anophagefferens* in some of the samples, giving false-positive identifications. This seems unlikely, however, since 46 species from five algal classes, including 20 chrysophytes, were tested when the antibody was first developed (Anderson *et al.*, 1989) and the dilution of the antiserum was adjusted

to levels that eliminate all but very specific antigen/antibody interactions. In addition, the size, shape and fluorescent labeling pattern 'halo' of the cells were all taken into account in the identification and enumeration.

One should also recognize that the surveys only provide a general picture of *A.anophagefferens* distributions within a window of time during the summers of 1988 and 1990. Sampling times were chosen to coincide with warm summer months when brown tides of this species are commonly observed, but it is possible that samples at other times of the year might have given a different distributional picture. Furthermore, in a survey of this magnitude, with samples being collected by different individuals at different times, the synoptic view suggested by Figures 1 and 2 could be somewhat misleading. Nevertheless, the distributional patterns of the two surveys are qualitatively quite similar, even though they were conducted 2 years apart.

Further resolution of the natural habitat and population distribution of *A.anophagefferens* will require a continuation of the use of this technique to screen new plankton samples from locations within and without our study area, as well as examination of archived material for the presence of this small, but potentially harmful chrysophyte. In this context, it is of note that this antibody and immunofluorescent technique were recently used to screen cells from a persistent brown tide in southern Texas caused by a small unidentified picoplankter. The immunoassay was negative for *A.anophagefferens*, a finding later confirmed by pigment analysis (Stockwell *et al.*, 1993). In the coming years, it will be interesting and informative to examine plankton material from other parts of the world to ascertain the global distribution of *A.anophagefferens*. The sensitivity and specificity of the immunofluorescent method, combined with its effectiveness on preserved samples, suggests that such studies are indeed possible.

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References

Anderson,D.M., Kulis,D.M. and Cosper,E.M. (1989) Immunofluorescent detection of the brown tide organism *Aureococcus anophagefferens*. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J.

(eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 213–228.

Bricelj,V.M. and Kuenstner,S. (1989) Effects of the 'brown tide' on the feeding, physiology and growth of juvenile and adult bay scallops and mussels. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 491–509.

Campbell,L. and Carpenter,E.J. (1987) Characterization of phycoerythrin-containing *Synechococcus* spp. by immunofluorescence. *J. Plankton Res.*, **9**, 1167–1181.

Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds) (1989a) *Novel Phytoplankton Blooms: Causes and Effects of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin.

Cosper,E.M., Dennison,W., Milligan,A., Carpenter,E.J., Lee,C., Holzapfel,J. and Milanese,L. (1989b) An examination of the environmental factors important to initiating and sustaining 'brown tide' blooms. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 317–340.

Dennison,W.C., Marshall,G.J. and Wigand,C. (1989) Effect of 'brown tide' shading on eelgrass (*Zostera marina* L.) distributions. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 675–692.

Hardy,C.D. (1976) A preliminary description of the Peconic Bay Estuary. Marine Sciences Research Center Special Report No. 3, State University of New York, Stony Brook, NY.

Keller,M.D. and Guillard,R.R.L. (1985) Factors significant to marine dinoflagellate culture. In Anderson,D.M., White,A.W. and Baden,D.G. (eds), *Toxic Dinoflagellates*. Elsevier, New York, pp. 113–116.

Nuzzi,R. and Waters,R.M. (1989) The spatial and temporal distribution of 'brown tide' in eastern Long Island. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 117–138.

Olsen,P.S. (1989) Development and distribution of a brown-water algal bloom in Barnegat Bay, New Jersey with perspective on resources and other red tides in the region. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 189–213.

Pritchard,D.W. and Gomez-Reyes,E. (1986) A study of the effects of inlet dimensions on salinity distributions in Great South Bay. Marine Sciences Research Center Special Report No. 70, SUNY, Stony Brook, NY.

Shapiro,L.P., Campbell,L. and Haugen,E.M. (1989) Immunochemical recognition of phytoplankton species. *Mar. Ecol. Prog. Ser.*, **57**, 219–224.

Sieburth,J.McN. and Johnson,P.W. (1989) Picoplankton ultrastructure: a decade of preparation for the brown tide alga, *Aureococcus anophagefferens*. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 1–21.

Sieburth,J.McN., Johnson,P.W. and Hargraves,P.E. (1988) Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae): the dominant picoplankter during a bloom in Narragansett Bay. *J. Phycol.*, **24**, 416–425.

Stockwell,D.A., Buskey,E.J. and Whitledge,T.E. (1993) Studies on conditions conclusive to the development and maintenance of a persistent 'brown tide' in the Laguna Madra, Texas. In Smayda,T.J. and Shimizu,Y. (eds), *Toxic Phytoplankton Blooms in the Sea*. Proceedings of the 5th International Conference, Elsevier, Amsterdam, in press.

Tracey,G.A. (1988) Feeding reduction, reproductive failure, and mortality in *Mytilus edulis* during the 1985 'brown tide' in Narragansett Bay, Rhode Island. *Mar. Ecol. Prog. Ser.*, **50**, 73–81.

Vieira,M.E.C. (1989) The case for meteorologically driven fluctuations in residence times of Long Island waters subject to Algal Blooms. In Cosper,E.M., Bricelj,V.M. and Carpenter,E.J. (eds), *Novel Phytoplankton Blooms: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms*. Springer-Verlag, Berlin, pp. 295–306.

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